Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

LEUCOCYTE STANDARDS AND THE LEUCOCYTE CONTENT OF MILKS FROM APPARENTLY HEALTHY COWS.

H. L. RUSSELL AND CONRAD HOFFMANN.
University of Wisconsin.

OF late years an increasing amount of attention has been given to the determination of the sanitary properties of milk supplies. The old standards, based largely upon the sense of smell and taste, are no longer regarded as the ultimate basis for a proper judgment as to the quality of milk. Various new methods have been introduced for the purpose of controlling the character of these supplies. Those so far proposed, however, have been developed for the detection of two different sets of conditions:

(1) The determination of the presence of organisms that have found their way into milk mainly through careless methods of handling.

(2) The detection of abnormal conditions in milk arising from its condition in the udder.

The first type of standards embraces those relating to the degree of cleanliness with which the milk is handled; the second concerns the healthfulness of the milk as it comes from the cow.

Among the more important standards that have been proposed may be mentioned, under Group (1), the following:

(*a*) The determination of the total germ content by cultural methods, a method which has been adopted by a considerable number of cities.

(*b*) The determination of the total germ content by direct microscopic examination, as proposed by Dr. Slack of the Boston Board of Health.

(*c*) The use of the Wisconsin curd test, by means of which is recognized the presence of gas-producing and taint-forming bacteria, organisms associated with careless methods of milk-handling.

Under Group (2) are included such test methods as reveal—

(*a*) The presence of special types of organisms, such as streptococci, that are presumed to be associated with the production of certain udder diseases.

(*b*) The supposed presence of pus, by the determination of pus cells, or the detection of associated elements, such as fibrin.

Each of these methods that have been suggested has elements of value, but in the present state of development, it is a question whether any absolute standard can be proposed that is a satisfactory basis for milk inspection. The actual detection of disease-producing organisms in milk is exceedingly difficult under most conditions. Fortunately there are not many diseases which occur in the cow that affect man. Tuberculosis is by far the most important of this class.

On the other hand, cattle not infrequently suffer from udder troubles—gargets of various types, in which inflammatory processes of varying degrees of severity may occur. In some cases these may be so slight as apparently not to affect the nature of the milk. Often, however, the milk becomes viscous, sometimes clotted or stringy, and may even be of a bloody nature. Occasionally such troubles as these develop into a gangrenous stage, in which pus is actually present in such quantities as to be easily recognizable. Milks of this character should, of course, be excluded from food supplies. In these cases, where a physical examination reveals an abnormal condition, a microscopic study naturally presents a far different picture from that which obtains in apparently normal milk. One of the prominent microscopic features in the case of milk that is abnormal at time of withdrawal is the presence of an unusually large number of certain blood elements, known as the white blood corpuscles, or leucocytes. In case pus is at all observable, these cellular elements are present in enormous numbers. Generally they can be detected, even where no physical signs of pus are apparent. The fact that milks known to be associated with abnormal conditions in the cow often contain these elements in such large numbers has led to the suggestion that all milks rich in leucocytes should be regarded with more or less suspicion.

Histologically, it is not an easy matter to differentiate between pus cells and the normal leucocytes, or white blood corpuscles. These latter cellular elements are, of course, normal constituents of blood, and are also found in variable quantities in milk supplies. It is quite probable that some of these cellular elements found in milk are derived from the gland tissues and are not leucocytes, although, upon staining, the majority of the cells are polymorphonuclear. Thus far, emphasis has been placed on the quantitative rather than the qualitative relation of these cells in milk. Whatever may be their nature, they are at present considered as associated with pus, if they are found abundantly in the milk.

The presence of a large number of leucocytes, either in blood or milk, is regarded as indicating an irritation of an inflammatory character, but the important question is, what constitutes an abnormal number? How many leucocytes can be present in milk before it should be considered that suppuration has taken place? The standards, so far chosen, have been more or less arbitrarily selected on what appears as rather inadequate data, and, from a comparison of results, it is apparent that much more comparative work needs to be made. Even the technical methods by which the leucocytes are determined do not show harmonious and constant results, and the interpretations placed upon the data obtained vary still more.

The formulation of these standards for milk inspection has already led to much dissatisfaction and complaint on the part of the dairy interests. In a number of cases, even certified supplies which have been handled with great care have been rejected on account of the high leucocyte content found in them, although the closest veterinary inspection failed to reveal the presence of any udder abnormality. We quote from a recent correspondent in Tennessee:

> The city chemist of Memphis is taking steps to condemn 35 to 75 per cent of the dairy cows of this county for alleged discovery of pus in the milk. He has condemned 16 out of 35 in our herd, and in the case of some dairymen, 90 per cent of their herds. The cows are in good condition and the veterinarian from the University of Tennessee has examined my herd, and pronounced their general condition good, and their udders perfect. There is absolutely no sign of inflammation.

In a letter from Doctor S. McC. Hamill of Philadelphia, who is the secretary of the Milk Commission of the Pediatric Society of that city, he says:

> Some of the best herds have been publicly condemned and brought into disrepute. It has not been shown by anyone that milk high in leucocytes has any injurious effect, nor has it been shown that their presence meant *per se* inflammatory changes in the udder.

As secretary of this commission, Doctor Hamill enters a vigorous protest against the attitude of the city board of health, which is enforcing a standard whereby milk supplies are condemned in which are found 100,000 or more leucocytes per c.c.

METHODS OF EXAMINATION.

Before discussing the standards proposed it is pertinent to study the methods of examination which so far have been devised.

Naturally the number of blood elements can best be determined by the use of the centrifuge. In the main, two methods have been suggested by which the leucocytes can be quantitatively determined; viz.:

(*a*) *Smeared-sediment method.*—This method, devised by Stokes[1] of the Baltimore Board of Health, and later somewhat modified by Stewart of the Philadelphia Bureau of Health, consists in centrifugalizing a definite quantity of milk in rubber-stoppered tubes, pouring off the cream and milk, rubbing up the collected sediment with water, and smearing this emulsion on a glass slide of definite dimensions. The preparation after staining is examined by a one-twelfth inch oil immersion lens, and the number of leucocytes per field noted.

(*b*) *Direct quantitative method.*—This method was suggested by Doane[2] and is carried out in essentially the same way as the generally accepted quantitative method of determining the blood cells. Ten c.c. of milk are centrifugalized in sedimentation tubes. The cream is then removed by means of a cotton swab and the material treated in the same manner a second time, thus wholly eliminating the fat.

The supernatant milk is then siphoned off. Water is added to the collected sediment, bringing the same to the one c.c. mark, and the whole thoroughly emulsified. Quantitative determinations are made from this material in a ruled blood counter (Thoma-Zeiss pattern).

Savage[3] has recently studied the relation of streptococci and leucocytes in milk and has employed a method quite similar to that of Doane. His technique was evidently developed independently, as he refers only to Stokes' method in discussing the subject.

Stokes' method has been adopted by most investigators who have studied this question. Bergey at Philadelphia has carried on quite an extensive series of examinations by this method, except that he substituted a cover-slip for the glass slide of given dimensions. The health boards of Philadelphia and Boston, the two cities that have been the most active in the examination of milks as to leucocytes, have both adopted this method.

The objections to this technical procedure are quite apparent. It is impossible to remove wholly the sediment from the rubber corks, and it is quite out of the question to distribute this material uniformly enough over the glass surface so that duplicate specimens can be secured which will give consistent results. This distribution can be more effectively obtained by emulsifying the sediment with a small quantity of water. Moreover, this treatment requires staining the cells, and if the leucocytes are not spread in an exceedingly thin layer, it is impossible to count them accurately. In our experiments there appears to be but little question as to the comparative merits of the two types of methods that have been proposed.

---

[1] "The Microscopic Examination of Milk," *Jour. of State Med.*, 1897, 5, p. 439.

[2] *Bull. 102, Maryland Agri. Expt. Sta.*, 1905 (often referred to in the literature as the Doane-Buckley method).

[3] *Jour. Hyg.*, 1906, 6, p. 123.

COMPARISON OF METHODS OF EXAMINATION.

In order to determine their relative accuracy, a considerable number of duplicate examinations were made upon the same sample of milk by the Doane-Buckley and the Stokes-Stewart methods. These data are incorporated in Table 1, from which it appears that the results obtained by the volumetric method (Doane-Buckley) are very much more harmonious than those secured by the smeared-sediment method. The average percentage variation in duplicate examinations in the smeared-sediment method is very great (112 per cent), while that of the other method rarely ever exceeds more than 10 to 20 per cent, and in 23 tests averaged less than 6 per cent.

TABLE 1.

COMPARATIVE EXAMINATION OF LEUCOCYTES PER C.C. IN MILK, AS DETERMINED BY THE TWO METHODS PROPOSED.

VOLUMETRIC (DOANE-BUCKLEY) METHOD					SMEARED-SEDIMENT (STEWART) METHOD			
Sample	First Examination	Second Examination	Average	Percentage Variation	First Examination	Second Examination	Average	Percentage Variation
1	590,625	700,000	645,310	18.0	403,200	238,500	320,850	69.0
2	325,000	288,750	306,875	12.0	16,200	113,400	64,800	587.0
3	1,012,500	975,000	993,750	3.7	99,100	154,000	126,560	55.4
4	11,250	12,500	11,875	11.1	4,500	16,200	10,360	260.
5	701,250	687,500	694,375	2.0	82,870	146,830	114,850	77.1
6	146,250	155,000	150,625	5.6	136,020	120,700	128,360	12.6
7	20,000	20,000	20,000	0.0	19,810	18,900	19,370	4.7
8	193,750	187,500	190,625	3.3	153,900	111,700	132,800	37.7
9	881,250	901,250	891,250	2.2	366,300	517,500	441,900	41.2
10	50,000	50,000	50,000	0.0	0	9,000	....	....
11	42,500	51,250	46,875	20.0	63,000	76,560	69,810	21.4
12	413,750	422,500	418,125	2.0	135,210	150,430	142,820	11.2
13	10,000	10,000	10,000	0.0	2,250	13,510	8,880	500.0
14	438,750	425,000	431,875	3.2	193,670	145,930	169,800	32.5
15	125,000	132,500	128,750	6.0	0	17,115	....	......
16	4,680	3,900	4,290	10.0	1,350	0	....	......
17	87,500	90,000	88,750	3.0	40,530	9,000	24,760	350.4
18	543,750	537,500	540,625	1.1	284,650	152,235	218,440	87.0
19	443,750	443,750	443,750	0.0	275,640	276,545	276,095	0.3
20	222,500	231,250	226,875	4.3	126,110	159,440	142,775	26.4
21	245,000	247,500	246,250	1.0	174,750	202,680	188,720	16.0
22	1,076,250	1,057,500	1,066,875	1.7	439,590	545,885	492,740	24.2
23	142,500	125,000	133,750	14.0	71,180	87,375	79,280	22.7
				Average, 5.6%				Average, 112%

It is noteworthy in the above that, quantitatively, higher results were almost uniformly obtained by the Doane-Buckley method than by the Stokes-Stewart method. This fact should be taken into consideration in the application of any standard.

Ward has also recently reported a comparison of these two methods,[1] in which similar results were obtained. From these data it appears, without question, that the volumetric method is very much more accurate for the determination of the actual conditions in milk; and not only in scientific studies on this question, but also in the practical application of any standard in public health work this should be given preference over the smeared-sediment method.

---

[1] *Rep. California State Board of Health, 1905–1906*, p. 142.

TECHNIQUE USED IN WORK HERE REPORTED.

The method which we have followed in the work to be reported is essentially the Doane-Buckley method with some slight modifications. A turbine Babcock tester was employed to centrifugalize 10 c.c. of milk in ordinary sedimentation tubes twenty minutes at 1,200 revolutions per minute. We have not followed the practice of remixing the cream and again centrifugalizing, as our experience showed so few leucocytes in the cream as to be practically negligible. Savage[1] found that the number of leucocytes in the supernatant fluid did not exceed 12 per cent of the total count.

The cream and the supernatant milk were removed, with the exception of the last half cubic centimeter, by aspirating with an exhaust pump, and wiping the walls of the tube with a cotton swab. The thoroughly mixed residue was then placed in the blood counter and examined unstained. While most observers have usually stained this material prior to examination, we have found no difficulty in working with unstained material. The cellular elements are easily differentiated, as they settle quickly to the bottom of the cell. The fat, which is a disturbing factor in such microscopic observations, is practically all removed in this process; the very few small globules which remain rise to the top of the liquid in the counting cell and do not interfere with microscopic observations.

The count is made with a No. 1 eye-piece, and a $\frac{1}{8}$-inch objective. Where the number of leucocytes exceeded 12 or 15 per microscopic field, six sets of 16 each of the smallest ruled squares of the blood counter, or approximately one-fourth of the entire area of the ruled scale, were counted. Where it was less than this, 10 to 16 such sets were counted. The average was then obtained, which, when multiplied by the factor 12,500 (the ratio between the counted volume and one c.c.), gave the total number of leucocytes per c.c.

FORMULATION OF STANDARDS AS TO PUS CONTENT.

Various numerical standards have been suggested from time to time as indicating the presence of pus in milk. A comparison of these shows that considerable variation exists, not only in the standards themselves, but in the interpretation of results.

Stokes[2] reached the conclusion that pus was present when more than ten[3] cells were found in a $\frac{1}{12}$-inch field. Bergey[4] adopted this same standard (10 per field) in 1900,[1] and his later studies have confirmed his opinion as to the correctness of this standard.

---

[1] *Loc. cit.*, p. 129.

[2] *Rep. Health Department, Baltimore, 1899;* also *Med. News*, 1897, 71, p. 45.

[3] Since then, according to Doane (*Bull. 102, Maryland Agri. Expt. Sta.*, p. 214), Stokes has suggested the advisability of increasing this limit to 25 leucocytes for the same field.

[4] *Univ. of Pa. Med. Bull.*, July–Aug., 1904, p. 2.

Stewart[2] of Philadelphia has increased his standard considerably above the limits selected by Bergey. He recommends spreading the sediment over one sq. cm. and advises condemnation of the sample when the microscopic field (with $\frac{1}{12}$-inch objective and No. 3 eye-piece) contains more than 23 leucocytes per field. As it requires practically 4,400 fields to cover the one sq. cm. of the smeared preparation, this would be equivalent to 100,000 leucocytes per c.c. This standard has been quite widely accepted and is now in force in a number of cities.

Doane, using his volumetric method, suggested a materially higher standard as he found quite frequently that the milk of apparently healthy animals exceeded the 100,000 limit. He suggested 500,000, and possibly 1,000,000 leucocytes per c.c., as a preferable limit.

As pointed out above, and also by Ward, the results obtained by the Doane-Buckley method how on the same sample of milk a very much higher leucocyte content than those obtained by the smeared-sediment method. It is, therefore, very necessary that a uniform method of examination be selected before adopting any standard for comparison.

RESULTS OBTAINED IN PRACTICAL WORK ON MILK EXAMINATION.

For the last two years certain milk supplies received in Philadelphia and Boston, have been examined as to numerical leucocyte content, the presence of streptococci, and total bacterial content.

Stewart has investigated six of the best milk supplies furnished in Philadelphia—supplies that were certified by the Milk Commission of the Pediatric Society of that city, with the following results:

TALBE 2.

RESULTS OF EXAMINATION OF CERTIFIED MILK SUPPLIES OF PHILADELPHIA BY LEUCOCYTE STANDARDS (STEWART).

Herd Number	Total Number of Samples Examined	Number of Samples containing Pus in Excess of Legal Standard (100,000 per c.c.)	Percentage of Samples Condemned on Pus Standards
1	54	7	13
2	16	4	25
3	54	2	4
4	54	4	8
5	52	4	8
6	52	0	0

Slack (*Thirty-fourth Health Report*, Health Department, Boston, 1905) gives the results of an inspection of the regular supplies furnished Boston, in which 5,559 samples were tested, and 279 (or 5 per cent) of them were condemned as containing pus in such quantities as to be considered as infected. Presumably this condemnation was made on the basis of an excess of 100,000 leucocytes per c.c., although specific information as to this point is not presented. In this case, the method of examination was the slight modification of that used by Stokes and Stewart.

---

[1] Reference to Bergey's original paper, published in the *Report of Secretary of Agriculture of Pennsylvania, 1900*, p. 123, shows that he proposed a limit of five leucocytes per $\frac{1}{12}$-inch field, and any excess of this number he believed to be associated with a pathological condition of the udder, especially if the leucocytes showed a tendency to be bunched together.

[2] *Amer. Med.*, 1905, 9, p. 486.

Savage, using the direct quantitative method of enumeration, examined the milk of 11 herds, or a total of 40 cows, in which in no case was there any physical symptom of udder infection. The range in leucocyte content found by him was 35,000 to 4,380,000 per c.c. Thirty-three of the 40 cows had 100,000 leucocytes per c.c., or over. An examination by him of the milk supplies taken from 17 different herds showed a range from 21,000 to 1,980,000 leucocytes per c.c. All but three herds showed a leucocyte content in excess of the 100,000 limit.

Doane has made a careful study by quantitative methods of the Station herd at the Maryland Experiment Station. Twenty-five animals were examined three times during a period of about three months. The first examination showed an excess of 100,000 leucocytes per c.c. in 14 out of 23 cases; the second examination, in 17 out of 22; and the third (two months later), in 10 out of 16 cases.

Another well-kept dairy herd near Baltimore was also examined by him and, in a single test made on 102 cows, the leucocyte content ranged from 2,000 to 4,600,000 per c.c., with an average of over 240,000. Forty-six animals in this herd showed an excess of 100,000.

It would appear from these figures that the milk of healthy animals often contains a much larger number of leucocytes than has heretofore been considered as permissible in a wholesome milk supply, but it is highly desirable that further data be accumulated, especially on individual cows whose physical condition could be more closely controlled.

RESULTS ON UNIVERSITY HERD.

No study has yet been reported on milks taken from individual animals where the examination has extended throughout a considerable consecutive period of time. Data of this sort would seem to be necessary in order to secure the normal individual range in leucocytes. The examinations which are here reported were made on samples taken from the University herd, which is composed of high-grade and pure-bred animals of representative dairy breeds. Daily examinations from the mixed-pail milk for a period of about one month were made during the summer (June and July), and then on the same animals for about two months in the fall (October and November).

This herd has been given a much closer degree of supervision than is ordinarily the case. The cows were carefully handled as to feeding, and also subjected to frequent veterinary inspection. The herd, with but a single exception, was in apparently good condition. This cow (No. 3) had chronic mammitis in one quarter.

TABLE 3.

LEUCOCYTE CONTENT OF MILK OF INDIVIDUAL COWS IN UNI

No. of Animal	Condition of Animal	June, 1906						July, 1906							
		24	25	26	27	28	29	2	5	12	13	15	16	17	19
2	Normal	37	128	....	....	....	131	....	64	31	....	29	31	35	51
8	“	....	....	5	....	....	16	....	22	1	....	....	10	25	64
9	“	....	....	1,097	....	....	306	297	....	260	382	....	501	176	320
10	“	....	39	....	....	....	....	60	....	4	....	26	22	26	232
11	“	....	115	....	....	....	....	162	....	139	....	120	54	57	131
12	“	....	....	66	....	....	....	37	....	35	....	12	12	44	56
14	“	272	....	208	....	137	....	204	....	439	372	....	456	260	....
15	“	....	....	159	....	224	....	376	....	141	297	....	306	129	254
16	“	103	61	....	47	....	....	....	55	200	67	....	44	82	201
19	“	896	....	1,026	....	35	....	1,707	....	314	1,100	....	397	641	866
20	“	....	....	122	....	....	45	87	....	95	156	....	187	141	129
22	“	....	82	....	....	....	....	56	....	19	76	....	79	16	41
24	“	....	181	....	....	186	....	92	....	214	125	....	297	39	141
26	“	....	4	....	23	82	....	....	....	....	....	7	12	6	....
27	“	58	101	....	80	59	....	81	....	350	....	164	329	194	157
28	“	....	....	....	....	....	....	....	....	....	....	....	....	....	....
29	“	....	....	....	....	....	....	....	....	....	....	....	....	....	....
30	“	....	....	....	....	....	....	....	....	....	....	....	....	....	....
1	Slight induration	11	....	115	124	....	56	....	356	162	....	226	162	235	539
3	Bad; acute mammitis	....	....	36	....	....	16	....	32	32	....	51	25	57	56
4	Udder coarse and hard	....	405	....	21	....	564	....	669	....	....	229	501	....	329
5	Slight induration	932	....	205	....	222	....	....	645	320	694	....	....	135	257
6	Indigestion	304	544	....	....	....	....	214	....	275	75	....	194	157	191
7	Slight induration	....	....	60	....	77	....	63	....	79	66	....	81	56	41
13	“ “	....	47	....	39	....	32	41	20	10	....	45	107	22	90
17	“ “	....	....	279	1.367	....	707	....	1,281	1,482	....	325	....	795	1,162
18	“ “	110	427	....	227	134	....	....	312	....	464	....	357	195	417
21	“ “	....	468	....	....	....	....	326	....	185	....	331	1,581	400	295
23	“ “	....	....	75	4,132	....	600	....	657	589	....	1,085	804	441	660
25	“ “	1,068	....	....	133	....	....	....	296	451	616	....	81	129	254

No. of Animals	Condition of Animal	October, 1906												
		9	10	11	15	16	17	18	19	20	21	22	23	24
2	Normal	....	....	156	92	....	172	135	86	147	75	32	121	59
8	“	11	9	9	21	5	....	....	....	....	....	....	....	....
9	“	941	472	576	....	1 966	712	1.037	400	387	126	864	307	241
10	“	45	22	166	44	69	339	150	81	120	90	64	169	67
11	“	20	61	47	20	57	55	35	101	59	99	50	111	100
12	“	....	....	....	70	26	50	75	145	46	87	132	90	89
14	“	345	....	....	....	....	339	892	304	262	362	597	301	169
15	“	....	....	6	19	....	....	....	....	....	....	....	....	....
16	“	....	....	....	....	....	....	....	....	....	....	....	....	....
19	“	381	397	275	239	204	347	557	370	581	264	185	664	1,066
20	“	....	139	254	69	139	212	401	175	91	166	11	137	131
22	“	....	....	....	....	....	....	....	....	....	....	....	....	....
24	“	147	119	187	54	181	162	232	179	135	75	52	46	169
26	“	30	36	112	30	47	24	30	19	21	21	27	12	12
27	“	350	2,191	1,416	1,087	2,014	....	....	....	....	....	....	....	....
28	“	....	....	....	....	....	30	39	30	42	201	34	36	74
29	“	....	....	....	....	....	24	47	57	32	12	15	15	16
30	“	....	....	....	....	....	12	25	14	24	41	41	12	46
1	“	....	....	156	92	....	170	189	89	175	99	182	79	269
3	Slight induration	....	489	912	3,475	3,465	3,625	1,097	1,501	1.350	1,044	1,041	....	....
4	Bad; acute mammitis	237	304	3.945	4,952	295	....	....	....	....	....	....	....	....
5	Udder coarse and hard	362	312	122	407	664	412	785	689	1,781	581	245	494	432
6	Slight induration	91	....	225	179	220	....	....	....	....	....	....	....	....
7	Indigestion	11	57	20	....	17	7	231	41	42	82	30	17	37
13	Slight induration	107	....	62	294	172	109	66	41	35	501	1,157	970	654
17	“ “	1,420	1,676	1,756	4,232	514	1,200	1,487	591	872	595	1,166	685	3,650
18	“ “	....	1,101	1.801	160	182	89	122	87	194	160	107	94	156
21	“ “	....	....	147	56	145	....	....	....	....	....	....	....	....
23	“ “	62	414	172	1,595	704	....	....	....	....	....	....	....	....
25	“ “	9	....	144	50	50	104	84	44	132	39	62	46	62
	“ “													

TABLE 3.

K of Individual Cows in University Herd (000 Omitted).

Y, 1906				September, 1906					October, 1906						Remarks
15	16	17	19	24	25	26	27	28	1	2	3	4	5	8	
29	31	35	51	....	144	....	60	99	139	135	....	200	194	....	In heat July 15, '06
....	10	25	64	39	16	26	19	12	10	....	....	9	....	46	
....	501	176	320	426	322	562	594	270	197	....	....	....	....	444	Calved June 23, '06
26	22	26	232	204	74	102	212	144	87	....	....	....	....	72	Calved September 14, '05
120	54	57	131	160	64	76	87	90	132	....	....	....	....	57	Calved May 3, '06
12	12	44	56	64	72	216	15	27	11	....	....	35	106	....	
....	456	260	....	550	629	644	531	....	175	....	....	201	189	....	Calved April 5, '06
....	306	129	254	304	341	137	132	....	....	....	....	....	....	....	
....	44	82	201	41	....	126	65	216	....	....	56	117	57	....	
....	397	641	866	....	....	....	....	....	....	....	....	551	595	235	Gargety for a time in 1905 Calved October 1, '06
....	187	141	129	401	85	139	166	208	65	50	....	110	141	151	
....	79	16	41	....	....	....	....	....	....	....	....	....	....	....	
....	297	39	141	64	85	82	60	72	....	141	....	....	....	87	Milk fever June 12, '06
7	12	6	....	35	31	8	25	29	21	14	....	....	....	42	
164	329	194	157	731	419	381	491	714	....	....	1,594	1,056	1.000	....	
....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	Calved August 21, '06
....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	Calved July 7, '06
....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	Calved September 18, '06
226	162	235	539	....	144	....	60	99	139	135	....	200	194	....	
51	25	57	56	107	206	172	125	145	2,267	....	....	1,287	1,104	669	
229	501	....	329	435	722	994	212	1,639	1,420	....	....	....	....	607	Aborted March 10, '06
....	....	135	257	682	207	282	316	797	166	....	....	....	....	275	Calved May 2, '06
....	194	157	191	210	187	169	100	407	....	157	....	160	....	....	
....	81	56	41	66	51	....	72	66	11	229	....	....	....	10	Calved March 17, '06
45	107	22	90	541	831	350	244	245	36	102	49	630	1,191	320	
325	....	795	1,162	854	326	566	395	972	150	410	....	....	....	1,266	Calved April 26, '06
....	357	195	417	....	....	....	....	....	....	....	....	....	....	....	Calved October 3, '06. Had milk-fever soon after
331	1,581	400	295	106	175	82	74	189	....	....	....	....	....	....	
1,085	804	441	660	379	197	94	144	364	100	....	....	441	154	....	
....	81	129	254	79	139	....	56	102	32	67	30	....	....	87	Calved September 18, '06

October, 1906												November, 1906			Remarks
21	22	23	24	25	26	27	28	29	30	31A.M	31P.M.	1	2	3	
75	32	121	59	77	212	157	76	222	243	229	162	210	170	404	
....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	
126	864	307	241	344	560	1,060	525	262	750	531	310	791	491	564	
90	64	169	67	70	65	36	66	145	69	91	157	166	54	162	
99	50	111	100	137	102	55	137	77	41	71	86	79	77	107	
87	132	90	89	65	182	150	117	82	172	124	157	105	117	172	
362	597	301	169	419	222	276	341	619	229	291	204	300	194	164	
....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	
....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	
264	185	664	1,066	347	610	366	294	1,214	581	481	335	981	512	614	
166	11	137	131	216	130	297	231	132	169	412	97	162	176	231	
....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	
75	52	46	169	114	136	92	99	137	91	125	61	59	92	115	
21	27	12	12	22	42	24	12	29	41	26	41	46	30	21	In heat October 12 and October 30, '06
....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	
201	34	36	74	44	110	35	17	61	76	54	44	132	46	36	
12	15	15	16	19	15	19	12	21	19	73	20	49	27	37	
41	41	12	46	17	10	26	16	30	11	15	3	19	26	20	
99	182	79	269	175	151	187	210	167	250	194	150	125	142	162	
1,044	1,041	....	....	....	....	....	....	....	....	....	....	....	....	....	
....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	
581	245	494	432	279	389	1.150	354	395	354	237	351	281	390	976	
....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	
82	30	17	37	60	109	39	26	137	54	62	65	106	45	79	
501	1,157	970	654	232	275	49	52	49	....	51	30	46	25	55	
595	1,166	685	3.650	841	1,329	1.281	1,266	2.451	3.627	822	510	307	494	1.225	
160	107	94	156	67	75	34	32	115	110	81	191	170	87	120	
....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	
....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	Gargety during October, '06
39	62	46	62	65	60	51	31	172	135	185	115	350	110	125	

In order, however, to ascertain with especial care the exact condition of each animal, as to the quality and condition of her udder, the entire herd was subjected to a most rigid examination by Dr. Alexander, the Station veterinarian, and Professor Humphrey, of the Animal Husbandry Department. In this examination every abnormality, however slight, was taken into consideration. We have therefore divided the results reported below on the basis of this physical examination, including in one group all those animals which were wholly free from any udder blemish whatsoever, while in the second group are included those in which some manifestation of present or past udder trouble was observable. In this latter group, the induration often was very slight, but the previous history of the animal usually indicated that such animals had had some form of udder disturbance at some earlier date. At the time of the present examination the milk of these cows showed no abnormal condition, except in the single case mentioned. This history is especially interesting, as it may throw some light on the cause of the high leucocyte content of apparently healthy cows.

The detailed results are herewith presented in Table 3 in which the daily observations are recorded in full, so that the same may be critically studied by other observers. These data can be comprehended in their entirety more readily, in graphical, rather than tabular, form, and in Fig. 1 they are therefore reproduced.

In Table 4 is condensed a summary of the foregoing results, in which the maximum and the minimum, as well as the average leucocyte content, is given; also the various observations are grouped as to the respective number of times in which the leucocyte content came within certain specified limits.

In discussing these results the herd will be separated into two sections:

1. Cows which show an entire freedom from all udder troubles, either past or present.

2. Cows showing upon careful physical examination a slight induration or otherwise abnormal condition of the udder, but no unusual condition as to character of their milk supply.

Of the 30 animals in the herd, only one (No. 3) at the time these examinations were made showed any symptoms of active udder

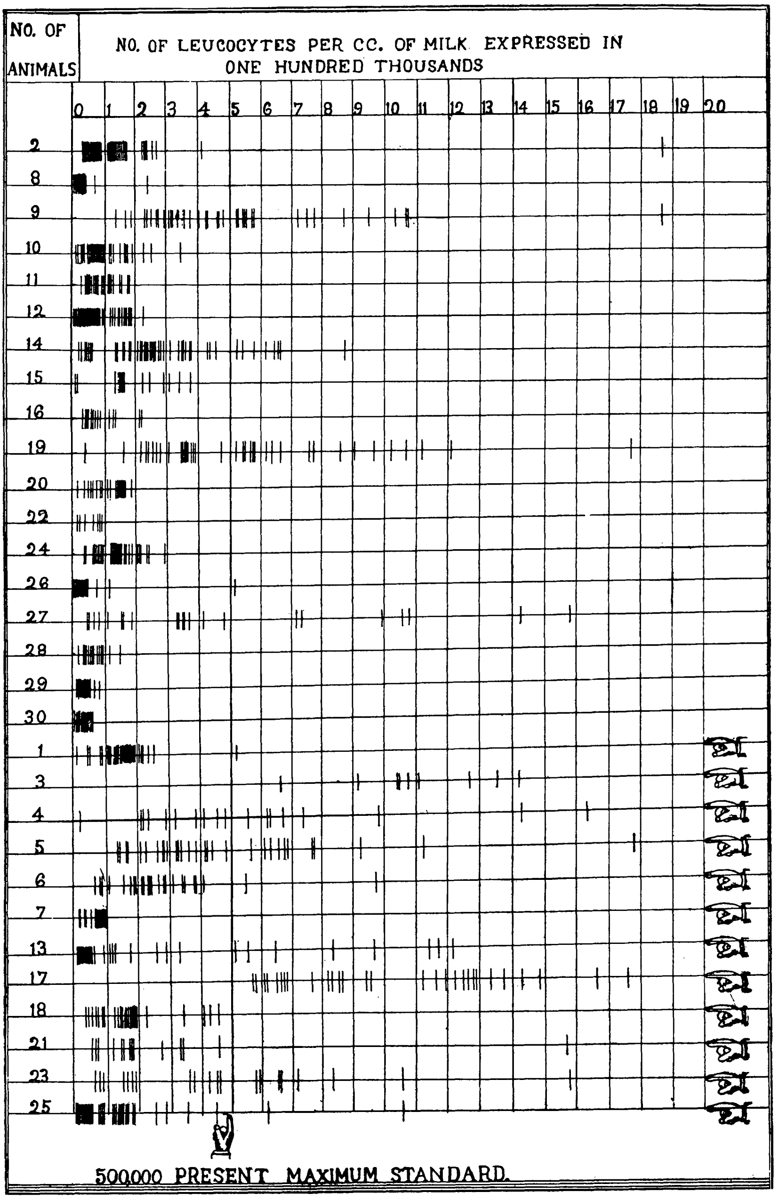

FIG. 1.—Leucocyte content of milks from University herd. Each determination represented by short vertical line. Animals marked with hand showed udder abnormalities. Note relative proportion of cases in excess of the present maximum standard.

trouble. In this case the milk was bloody and often clotted. The milk of the remaining 29 animals was perfectly normal in every respect, not only on the basis of its physical appearance, but by chemical and bacteriological analysis as well.

TABLE 4.

SUMMARY OF DATA IN TABLE 3.

Group 1. Perfectly Healthy Animals.

No.	CONDITION	NO. OF TESTS	MAXIMUM COUNT	MINIMUM COUNT	AVERAGE OF ALL TESTS	NO. TIMES LEUCOCYTE CONTENT PER C.C. FELL WITHIN FOLLOWING LIMITS				
						50.000 or Below	From 50.000 to 100.000	From 100.000 to 500,000	From 500.000 to 1.000.000	1,000,000 and Above
2	Normal	38	403.750	26.250	121.175	8	10	20	..	
8	"	20	63.750	1,250	18 815	19	1	..	..	..
9	"	38	1 966.250	126,250	536.280	..	..	21	13	4
10	"	38	338,750	3.750	100.400	8	15	14	..	..
11	"	38	162.500	20.000	85,130	5	19	14	..	..
12	"	36	216.250	11,250	85.225	11	12	13	..	..
14	"	37	892.500	137,500	327.000	..	..	29	8	..
15	"	12	341,250	128,750	235.520	..	..	12	..	..
16	"	16	216.250	41.250	96.360	3	7	6	..	..
19	"	30	1,707.500	35.000	561.825	1	..	17	13	5
20	"	41	412.500	11.500	162.500	2	8	31	..	..
22	"	7	81.850	16.250	52,770	3	4	..	..	..
24	"	39	297.000	38,750	121.300	2	16	21	..	..
26	"	39	532,500	3.750	42.000	36	1	1	1	..
27	"	23	2,191.250	58.000	652.960	..	4	10	2	7
28	"	24	201,250	16.250	60.130	14	7	3	..	..
29	"	29	72,750	3,750	25.000	29	2	..	..	..
30	"	26	46.250	31.250	19.880	26	..	..	..	..
	Totals....................	537	....	......	......	167	106	212	37	16
	Percentage of total.........	...	......	......	......	31.1%	19.8%	39.4%	6.8%	2.9%

Group 2. Animals Having Udder Indurations of Varying Severity but Producing Apparently Normal Milk (Except No. 3).

1	Slight induration	38	538,750	11.250	167,725	1	7	29	1	..
3	Bad; chronic mammitis	27	3,625.000	16.250	903.380	5	3	6	2	11
4	Udder coarse and hard	19	4.952,500	21.300	972,700	1	..	8	6	4
5	Slight; one-quarter	39	1,781,250	122.500	476,520	..	..	27	10	2
6	Indiges.; normal otherwise	19	543,750	75.000	213,700	..	2	10	1	..
7	Slight; one-quarter	38	231,250	7.500	63.375	15	17	6	..	..
13	One-quarter harder	43	1,191,250	10.000	225.935	18	6	11	6	2
17	Slight; one-half of udder	40	4.232,500	150,000	1,274,115	..	..	8	13	19
18	One-quarter slight (?)	32	1.801.250	32.500	249.450	2	7	21	..	2
21	One-quarter harder	15	1,581.250	66.850	304,125	..	3	11	..	1
23	Slight in one-quarter	22	4,132.500	62.500	630.170	..	3	10	6	3
25	One-quarter affected	39	1,068.000	8,750	149.950	7	13	17	1	1
	Totals................	371	......	......	......	49	61	170	46	45
	Percentage of total.....	...	......	......	......	13.2%	16.4%	45.9%	12.4%	12.1%

RESULTS.

A study of the preceding tabular data shows some peculiar results. It is apparent that the leucocyte content of some of the cows was remarkably uniform, while in a considerable number of cases the results were widely variable. It is to be noted, however, that the more uniform results were found in the first section of the herd, in which no previous history of udder trouble was observed. Of the 18 out of the 29 animals which showed a leucocyte content that was quite constant on successive examination, 14 cases belonged to Group 1 and only four to Group 2. Of the 11 instances in which the leucocyte content was subject to wide fluctuations, only four belonged to Group 1 while seven were found in the second group in which there was a more or less marked induration in the udder.

This would seem to indicate that lower and more constant results are more likely to be obtained with animals in which no udder trouble had ever appeared than with those having any history of a past inflammatory condition.

It is of course hardly possible to average results that fluctuate so widely, and draw any satisfactory conclusions, but if the data are arranged, as in Table 4, on the basis of the number of times the leucocyte content fell into certain numerical groupings, it is evident that the cellular content of milks drawn from perfectly normal cows averages lower than in Group 2.

Doane has suggested 500,000 leucocytes per c.c. as a possible limit by his method. On the basis of this standard, the results of our examination would be as follows:

Eighteen animals in the perfectly healthy group showed in 537 tests a leucocyte content of 500,000 or less in 90 per cent of the cases, while the 11 animals, in which induration was more or less marked, in 371 tests showed a similar condition in 75 per cent. While it appears from this that milks of high leucocyte content were found with considerably greater frequency (approximately two and one-half times), in the case of cows showing a fibrous induration of the udder, still it is noteworthy that practically one-tenth of all examinations made on cows entirely normal and healthy in all respects showed a leucocyte content that was in excess of the highest standard yet established. In this group of perfectly healthy animals 16 cases (or 3 per cent) were observed in which the cellular content was in excess of even 1,000,000 cells per c.c.

In the second section of the herd two animals contributed 30 of the 45 observations in which more than 1,000,000 leucocytes per c.c. were found. One of these (No. 3) was the only animal in the herd that showed any acute udder trouble. No. 17, while showing no marked involvement of the udder, almost invariably had a high leucocyte content.

When these results are studied on the basis of whether they would pass the standards that have been proposed as a maximum limit, it appears that in many cases they would have been condemned. While it is impossible to compare accurately the results of the examination by the direct quantitative method with what would have been found by the smeared-sediment method, it is certain that a considerably higher standard would be necessary, if the Doane-Buckley method was the one in general use.

It is apparent from these studies that the leucocyte content of normal milk drawn from apparently normal animals is quite often so high that the milk would be classed as coming from diseased animals when judged by the standards that have heretofore been proposed. That such results are obtained from animals whose record has shown no clinical history of a diseased condition would signify that complete reliance cannot be placed upon quantitative leucocyte standards alone.

While it is undoubtedly true that cows suffering from udder trouble often do produce milk that is rich in leucocytes, it would seem equally true that similar quantitative results were found with such frequency in milks from perfectly healthy animals as to vitiate the accuracy of a test based solely on a numerical foundation. This being true, it seems necessary to modify the limits that have been adopted by a number of cities where the acceptance or rejection of a milk supply is based upon such standards as are here considered. As interpreted at present, they undoubtedly work injustice to the dairy interests, as milk supplies would be frequently condemned which come from animals in which no clinically recognized troubles can be ascertained.

Undoubtedly the leucocyte standard can be so formulated as to be of material service in the matter of milk inspection, but the question needs further study. So far as technique is concerned, the volumetric method (Doane-Buckley) is the more accurate. As to its applicability in routine inspection work, our experience is that this test can be made as rapidly as the smeared-sediment method, and is less trying on the eyes.